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Published in:
A C S Catalysis

Link to article, DOI:
10.1021/acscatal.7b00540

Publication date:
2017

Document Version
Peer reviewed version

Mechanism of Cobalamin-Mediated Reductive Dehalogenation of Chloroethylenes

Li Ji[*,1], *Chenchen Wang*[1], *Shujing Ji*[1], *Kasper P. Kepp*[*,2], *Piotr Paneth*[*,3]

[1] College of Environmental and Resource Sciences, Zhejiang University, Yuhangtang Road 866, Hangzhou 310058, China

[2] DTU Chemistry, Technical University of Denmark, Building 206, Kgs. Lyngby, DK-2800, Denmark

[3] Institute of Applied Radiation Chemistry, Faculty of Chemistry, Lodz University of Technology, Zeromskiego 116, 90-924 Lodz, Poland

Abstract

Reductive dehalogenation involving cobalamin has ~~been~~ proven~~d~~ ~~to be~~ a promising strategy for decontamination of polluted environments. However, cob(I)alamin can act both as a strong reductant and a powerful nucleophile, and thus several competing dehalogenation pathways may be involved. This work uses experimentally calibrated density functional theory on realistic cobalamin models to resolve controversies of cobalamin-mediated reduction of chloroethylenes by exploring mechanisms of electron transfer, nucleophilic substitution, and nucleophilic addition. The computational results provide~~s~~ molecular-level insight into the competing pathways for chloroethylenes reacting with cob(I)alamin: the computed ratios of inner-sphere to outer-sphere pathways for perchloroethylene and trichloroethylene are 17:1 and 3.5:1 respectively in accord with corresponding experimental ratios of > 10:1 and > 2.3:1, while the computed outer-sphere pathway for other less-chlorinated ethylenes is hampered by high barriers (> 25 kcal/mol). Thus, a new mechanistic picture ~~has been~~is obtained~~: that~~ T~~t~~he highly-chlorinated ethylenes primarily react via an inner-sphere nucleophilic-substitution pathway, ~~while~~ whereas the less -chlorinated ethylenes mainly react through an inner-sphere nucleophilic-addition pathway. ~~Especially, the~~ ~~e~~Quantitative comparison of standard reduction potentials ~~between~~ of the formed chlorinated -cobalamin and cob(II)alamin/cob(I)alamin couple can be used to distinguish whether the~~-~~ inner-sphere pathway ~~could~~ can proceed or not, and ~~the~~ linear free energy relationships have been developed to predict the reductive dehalogenation reactivity within a given mechanism. ~~Furthermore~~Finally, we ~~have~~ propose~~d~~ new dual isotope analyses for distinguishing the various environmental dehalogenation mechanisms.

KEYWORDS: reductive dehalogenation, cobalamin, ~~competing pathways,~~ density functional theory, ~~standard~~ reduction potential, inner-sphere pathway, dual isotope analyses

Introduction

The widespread industrial application of halogenated compounds as solvents, chemical intermediates and pesticides are of great environmental concern~~, with~~ due to ~~the~~ adverse effects on ecosystems and human health.[1-2] The negative effects of halogenated compounds are generally attributed to the halogen atoms~~;~~, thus, the transformation of halogenated compounds to less- or non-halogenated products is a promising remediation strategy.[3-4] Among various dehalogenation remediation strategies, cobalamin (vitamin B12) promoted dehalogenation reactions have garnered considerable attention, owing to evidence that the cell component responsible for dehalogenation reactions by several anaerobic bacteria is most likely this transition-metal coenzyme.[5]

Cobalamin is the largest by molecular mass and arguably the most complex (in terms of functional groups) cofactor in biology, consisting of a cobalt atom coordinated by four nitrogen atoms of the corrin ring, as shown in **Scheme 1**. Under non-reducing conditions, the cobalt atom commonly exists in the +3 oxidation state (cob(III)alamin), axially coordinating two ligands (methyl or cyanide group in the "upper" and 5,6-dimethylbenzimidazole (DMB) in the "lower").[6-7] In abiotic systems, cob(III)alamin can be reduced to 4-coordinated cob(I)alamin without axial ligands in the presence of strong reducing agent in aqueous media.~~,~~[8-9] ~~while this model~~ These model systems mimicking microbial dehalogenation ~~has~~ have been used in abiotic remediation strategies for treatment of contaminated field sites.[10] Compared with the rigorous selectivity of enzymatic systems, *in vitro* ~~studies have shown that~~ cob(I)alamin can catalyze nonspecific reductive dehalogenation of many halogenated compounds, such as chlorinated methanes, ethanes,

Commented [KPK1]: This refers to previous sentence, i.e. the cob(I)alamin. Is this correct?Otherwise specific which type of model systems

higher alkanes, ethylenes, arenes, etc.[11-12] ~~Thus~~Accordingly, the abiotically reductive dehalogenation by cobalamin has substantial potential for use in remediation approaches~~;~~,[10, 13-14] ~~while some~~ other transition metal complexes (e.g. iron porphyrin) as well as nano-scale zero-valent metal systems~~s~~ (e.g. iron, zinc, ~~et al.~~etc.) have also been reported to be ~~potentially good~~useful reductive dehalogenation catalysts.[15-17] Practically, degradation of halogenated compounds by reductive dehalogenation reactions may occur via numerous routes. In order to assess whether ~~the~~ dehalogenation brings about significant detoxification, the fundamental knowledge of the reductive dehalogenation mechanisms involving cobalamin is essential. However, cob(I)alamin contains the unusual combination of properties ~~that it is~~of being both a strong reductant~~,~~[18] and one of the most powerful nucleophiles that is ~10^4 times more nucleophilic than the Cl anion in S_N2 reactions,[19] which makes the reaction modes more diverse and complex.

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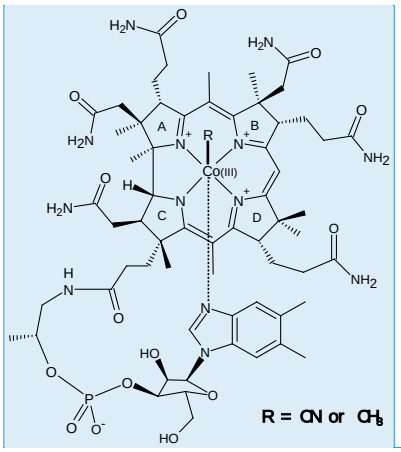
Scheme 1. Structure of the Cobalamin



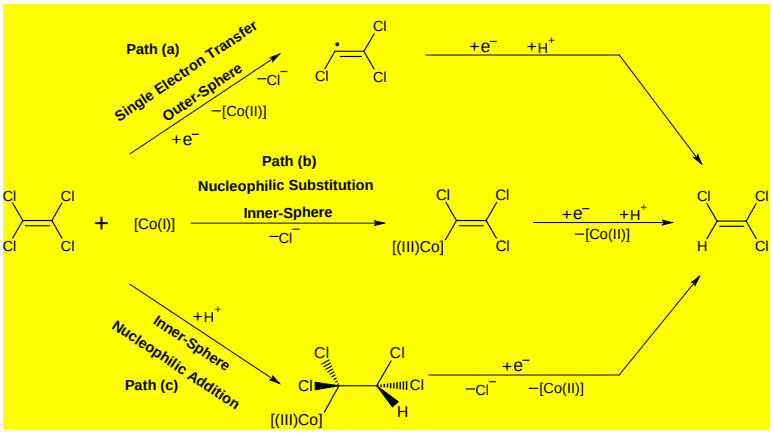
Commented [KPK3]: I think this should be a figure not a scheme – a scheme is e.g. a reaction process.

Among various halogenated compounds, the mechanistic study of cobalamin-~~-~~mediated reductive dehalogenation of chloroethylenes~~,~~ has attracted particular attention.[11] Cob(I)alamin has been reported to participate in the sequential dehalogenation of perchloroethylene (PCE), trichloroethylene (TCE), *cis*-1,2-dichloroethylene (*cis*-DCE) , *trans*-1,2-dichloroethylene (*trans*-DCE), and vinyl chloride (VC), resulting in the production of nontoxic ethylene.[9, 20-25] As shown in **Scheme 2**, the initial step for cob(I)alamin-catalyzed chloroethylenes can be summarized as an outer-sphere (reaction occurring between chemical species ~~with remaining~~in a non-connected state) or an inner-sphere (with a chemical bond forming between chemical species during the reaction) process, ~~which can be categorized in more detail~~more specifically as single electron-~~-~~transfer [outer-sphere (path a)], nucleophilic substitution [inner-sphere (path b)], and nucleophilic addition [inner-sphere (path c)] mechanisms. Most previous work focused on the reductive dehalogenation mechanism of highly -chlorinated substances, PCE and TCE.[9, 21, 23-25] On the basis of kinetic experiments, ~~the~~ pH-independent rate constants were observed, which may rule~~s~~ out inner-sphere nucleophilic addition ~~route~~ proceeding with simultaneous protonation.[21-22] Meanwhile, cob(I)alamin-mediated dehalogenation of PCE and TCE with increasing amounts of d_7-isopropanol, a D• donor, resulted in ~~as~~ at most 10% of the PCE-derived deuterated products and 30% of the TCE-derived deuterated products, in agreement with outer-sphere one-electron transfer.[21] However, this outer-sphere mechanism is not in accord with stereochemical result~~s~~ that ~~the~~ dehalogenation of TCE by cob(I)alamin produces greater amount of *cis*-DCE compared to *trans*-DCE (> 15:1), markedly different from the ratio obtained with identified electron-transfer reagents (< 5:1).[23] Support~~ing~~ for the nucleophilic substitution mechanism originated from experimental observation ~~that the~~of molecular mass consistent with dichlorovinylcobalamin ~~had been observed~~ in mass spectra during the TCE dehalogenation reaction.[25] If this nucleophilic

substitution mechanism works for PCE with cob(I)alamin as well, the trichlorovinylcobalamin from the PCE dehalogenation reaction should be detected, but this has not been the case.[11]

The experimental work performed with isopropyl alcohol-d_7 at different concentrations has showed that, in contrast to PCE and TCE, there ~~were not marked~~were only few ~~-~~deuterated products captured for the cobalamin-catalyzed reductive dehalogenation of less-chlorinated substances, *cis*-DCE, *trans*-DCE, and VC. ~~It~~ This indicates that there is not significant quantities~~y~~ of free radicals produced ~~in~~ during the dehalogenation process. Meanwhile, ~~the~~ kinetic experiments ~~have~~ shown~~n~~ that cobalamin reductively dehalogenate~~d~~ *cis*-DCE, *trans*-DCE, and VC in pH-dependent reactions.[22]

Scheme 2. Alternative Reaction Mechanisms for Reductive Dehalogenation of Chloroethylene Catalyzed by Cobalamin[a]



[a] taking PCE as an example

Isotope fractionation ~~in~~ during reductive dehalogenation of chloroethylenes with cobalamin has been investigated using compound specific isotope analysis (CSIA).[9, 26-31] CSIA ~~is able to~~

offers new insight into the organic pollutant degradation mechanism, especially when the competing reaction pathways are unknown.[32-33] In the process of cobalamin-mediated reductive dehalogenation of chloroethylenes, most previous work focused on stable carbon isotopes, and stable chlorine isotopes were not commonly studied ~~so often~~. For example, the reported carbon bulk isotope fractionation factors (ε_{bulkC}) are −15.8‰ for PCE[26] and −16.1‰ for TCE[9]; however, in contrast to cobalamin-catalyzed PCE and TCE, the reported values for *cis*-DCE (−25.5‰)[27] and VC (−31.1‰)[28] are much larger, which may indicate an entirely different reductive dehalogenation mechanism. Until now, no systematic computational investigation of isotope fractionation within the different operative pathways for reductive dehalogenation of chloroethylenes in the presence of cobalamin has been undertaken although such investigation should shed light onto this ongoing mechanistic debate.

Computational analysis of the catalytic mechanism can provide insight into the electronic structure features governing reaction mechanisms.[34-43] ~~which has already been performed to give~~Such studies have provided insight into the viability of various intermediates and pathways in the reductive dehalogenation of chloroethylenes with cobalamin[44-46] as well as synthetic cobaloximes.[47-48] ~~More specifically, t~~The computed electrochemical properties of the reduced chloroethylenes and chlorinated-cobalamins have been useful for interpreting some experimental observations, ~~such as~~e.g. indicating that the formed chlorinated vinyl radicals during reductive dehalogenation may be reduced to anionic forms competing with their rebound to cob(I)alamin to produce vinylcobalamins.[44] ~~It is noteworthy that~~Notably, most work has used the ~~cobalamin structure~~ simplified ~~through cutting off the~~corrin model without side chains and ~~replacing the axial DMB base with~~ imidazole instead of DMB~~for studying the mechanism of reductive dehalogenation~~, yet these substituents can affectcobalamin eelctronic structure substantially, so

Commented [KPK4]: I suggest citing my paper Kepp 2014 (reference 55 in the current version) where I study full cobalamins and show that these side chains are important.

~~while~~ the influence of omitting the substituents and ~~substitution of~~using the simpler axial base needs to be addressed. Until now, the precise reductive dehalogenation mechanism has not been established in details that would warrant explanation of all experimental observations collected so far.

In this work we use density functional theory (DFT) on realistic full cobalamin structures to address the following unsolved mechanistic questions: (i) why pH-independent rate constants have only been observed for the reactions of PCE and TCE with cobalamin, but not for cobalamin-catalyzed reactions of *cis*-DCE, *trans*-DCE, and VC; (ii) why there is conflicting evidence for the dehalogenation processes of cobalamin-mediated PCE and TCE favoring either the outer-sphere ~~pathway~~ or ~~the~~ inner-sphere pathway, respectively; (iii) why only dichlorovinylcobalamin has been detected ~~during~~ upon reaction of TCE with cobalamin, but not trichlorovinylcobalamin in the reaction of cobalamin-mediated PCE; (iv) whether the comparison between calculated kinetic isotope effects (KIEs) and experimental apparent kinetic isotope effects (AKIEs) can be used to identify the competing dehalogenation pathways.

Computational Methodology

~~**The System for Cobalamin Mediated Chloroethylenes**~~**Computational Details**. All calculations in this work were performed with the Gaussian 09 Revision D.01 program package.[70]

The complete 4-coordinated cob(I)alamin species (with the nucleotide loop ~~is clipped~~off), ~~was~~ were used as ~~initial structure~~basis for our computational work. The geometry optimizations and frequency analyses ~~have been~~were carried out in the gas phase using the Perdew-Burke-Ernzerhof (PBE) functional,[49-50] combined with Ahlrich's TZV basis set[51] for Co and 6-31G** basis set[52] for C, N, H, O and Cl (denoted ~~as~~ BSI). The PBE functional has been previously shown

Commented [KPK5]: ?

Has only dichlorovinylcobalamin been seen when TCE reacts with Cbl, and only trichjlorovinylcbl with PCE? This sentence is unclear

Commented [KPK6]: Header is unclear; "cobalamin-mediated chloroethylenes" simple suggestion made

to accurately reproduce~~s~~ the experimental frequencies of the corrin-based normal modes of vibration~~,~~[8] and ~~yields~~ geometries ~~in according to experimental cob(I)alamin structural parameters from XAS studies,~~[53-54] and accurate Co-C bond dissociation energies[55]. Subsequent frequency calculations were run to confirm that all ground states had only real frequencies, whereas the transition states had one imaginary frequency. The computed vibrational frequencies were further used to quantify the zero-point energy correction (ZPE)~~,~~ and enabled us to convert the electronic energy to the Gibbs free energy at 298.15 K and 101.325 kPa. ~~The i~~Intrinsic reaction coordinate (IRC) calculations were performed ~~further~~ to verify that the transition states ~~really~~ actually connected the reactants and products. Based on the PBE-optimized structures in the gas phase, the water solvation effects were calculated by COSMO continuum-solvation model (CPCM, dielectric constant = 78.3) at the PBE/BSI level of theory. The dispersion interactions were ~~considered through doing~~included via single-point calculations at the PBE-D3/BSI level of theory~~, due to the standard DFT method lacking of such interactions~~.[56] Unless otherwise specified, all relative free energies for cobalamin-mediated chloroethylenes reactions reported were based on PBE/BSI data including solvation and D3 dispersion corrections ($\Delta G + E_{solv} + E_{disp}$). We also performed PBE-D3 geometry optimizations with CPCM in water solution on five structures of cob(I)alamin, cob(II)alamin, base-off trichlorovinylcob(III)alamin, base-on trichlorovinylcob(III)alamin and base-on trichlorovinylcob(II)alamin to ~~verify~~ test the ~~reliance~~ reliability of the ~~above methods~~approach.~~,~~ ~~the results of which show that~~The results show that the effects of dispersion and solution ~~is negligible~~ on the optimized geometries are negligible (the geometrical comparison between PBE/BSI optimized structures in the gas phase and PBE-D3-CPCM/BSI optimized structures is shown in **Figure S9** ~~in~~ of the Supporting Information).

Commented [KPK7]: If this sentence is kept XAS should be defined

Commented [KPK8]: Cite instead the 2010 reference for Grimme's D3

Cobalamin chemistry is generally low-spin, as implied by the ground state of cob(I)alamin and cob(III)alamin with singlet state and cob(II)alamin with doublet state.[6, 8, 57] ~~As further proof, the calculations in this work at the~~Supporting this, the PBE/BSI ~~level of theory~~ free energy including solvation and D3 dispersion corrections ~~show~~ for cob(I)alamin and cob(III)alamin (base-off trichlorovinylcob(III)alamin) ~~on~~ reveal triplet state~~s~~ ~~are~~ at 21.3 and 18.4 kcal/mol ~~respectively~~ higher energy, respectively, than the~~ir~~ singlet state ~~specie~~s, while the cob(II)alamin ~~on high spin~~ quartet state is 29.1 kcal/mol ~~higher than its~~above the ~~low spin~~ doublet state ~~species~~; thus, in this work these species were all optimized in the low-spin state.

Particular attention ~~has been~~was focused on the electronic structure of cob(I)alamin. Previous computational studies have investigated the electronic structure of ~~four~~4-coordinated cob(I)alamin ~~without the axial DMB base~~ and ~~five~~5-coordinated cob(I)alamin with the axial DMB base using DFT and CASSCF calculations.[8, 19, 58] ~~The~~ TD-DFT calculations on truncated ~~four~~4-coordinated cob(I)alamin ~~have suggested~~suggest that ground state cob(I)alamin is purely a closed-shell singlet d^8 species.[8] ~~Subsequent~~ CASSCF calculations ~~on the truncated cob(I)alamin~~ have shown that the dominant contribution to the ground state wave function is the closed-shell singlet d^8 Co(I) configuration for ~~four~~ 4-coordinated cob(I)alamin~~,~~[19, 58] and for ~~five~~5-coordinated cob(I)alamin with ~~very~~ a weakly coordinated axial base,[58] while cob(I)alamin is mainly ~~dominantly the~~ open-shell singlet/triplet d^7 Co(II)-corrin $(\pi^*)^1$ diradical configuration with a strongly coordinated axial base.[58] ~~Then, t~~The reaction mechanism~~s~~ of methyl transfer between cob(I)alamin and CH_3–~~H_4Folate~~ –H_4folate catalyzed by methionine synthase ~~were revealed~~have been studied computationally, with the ~~four~~4-coordinated base-off cob(I)alamin conformation for studying the S_N2 pathway and the ~~five~~5-coordinated base-on cob(I)alamin conformation for studying the electron-transfer pathway, giving similar barriers for these two pathways.[36] However, while

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cob(I)alamin ~~only existing~~ in the enzyme can coordinate to the axial DMB base ~~due to the H-bonding between the base and nearby amino acid residues.~~. ~~The~~ X-ray absorption spectroscopy~~ic experiments~~[54] ~~as well as~~and DFT computations[8] have shown that cob(I)alamin in solution is not axially coordinated to DMB base at all, ~~which is proved again in this work that PBE calculations show the complete five-coordinated cob(I)alamin on open-shell singlet/triplet state is about 22 kcal/mol higher in free energy at PBE/BSI level of theory including solvation and D3 dispersion corrections than the complete four-coordinated cob(I)alamin on close-shell singlet state plus DMB base in solution.~~ Supporting the strict low-spin closed-shell configurations as found in our study. Furthermore, ~~in this work, the closed-shell~~the singlet stability of the ~~complete four~~4-coordinated cob(I)alamin was pro~~v~~ved by mixing the highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO) from PBE~~orbitals using PBE functional,~~ and ~~the~~ by CASSCF single-point calculation on the complete (not truncated) ~~four~~4-coordinated cob(I)alamin.

Many studies have ~~also~~ shown the experimentally calibrated PBE and BP86 methods are accurate for ground-state thermochemistry of cobalamins,[59] ~~partly because~~and the required active spaces from CASSCF e.g. CASPT2 methods are out of range to be computationally tractable, ~~and~~ partly because of the basis set requirements. ~~To summarize what has been mentioned above~~Accordingly, this work focuses on the ~~four-~~4coordinated cob(I)alamin ~~on~~ with the closed-shell singlet state using PBE ~~method~~ to study the reductive dehalogenation mechanisms.

Electrochemical Properties of Chlorinated Cobalamin. The aqueous-phase standard reduction potentials (E^0) referenced with respect to the standard calomel electrode (SCE) (E^0 vs. SCE, unit: V) of all chlorinated vinyl- and ethyl-cobalamins as well as the cob(II)alamin/cob(I)alamin couple were converted from the aqueous-phase adiabatic electron

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The new SI data are not discussed with references to Tables.

Right now only a few SI tables are mentioned.

Is it a journal policy that all SI tables and figures should be referenced in order? Some journals have that.

affinities (AEA~~).), while T~~t~~he absolute reduction potential of SCE for cobalamin ~~is~~ applied was 4.52 V ~~as recommended before~~,[60] as shown in eq 1:

$$E^0 \text{ vs. SCE (V)} = \text{AEA} - 4.52 \quad (1)$$

The AEA values were in the form of free energy changes~~, with~~ using the PBE single-point calculations ~~obtained from~~on the PBE/BSI-optimized geometries using the larger 6-311+G(2d,2p) basis set[61] for main group atoms and TZV for cobalt (denoted as BSII), including ~~water~~ solvation ~~energy~~ and ~~D3~~ dispersion corrections (BSII level of theory), and free energy corrections (BSI level of theory). The vertical electron affinities (VEA) for the base-off and base-on less-chlorinated ethylcobalamins were obtained from PBE/BSII//BSI single-point calculations with water solvation and D3 dispersion corrections. ~~Note f~~For cob(II)alamin and all chlorinated-cob(II)alamins ~~on~~ with doublet states, S^{**2} values after annihilation ~~are~~ range from 0.7500 to 0.7502, ~~thus~~ i.e. there are no spin contaminations of the wavefunctions ~~for all doublet cob(II)alamin~~of these species.

Electrochemical Properties and Electrophilic Reactivity of Chloroethylenes. All calculations for the electrochemical properties of chloroethylenes were performed using the PBE/aug-cc-pVTZ level of theory (BSIII) with CPCM solvation model of aqueous solution (dielectric constant = 78.3). VEA for all chloroethylenes are electronic energy differences in ~~aqueous-phase~~water, while adiabatic electron affinities for all vinyl radicals are ~~aqueous-phase~~ free energy change in water~~s~~. The adiabatic electron affinities of vinyl radicals were translated into aqueous-phase E^0 vs. SCE. The e~~E~~lectrophilic index (ω), ~~developed from~~based on the concept of ~~the~~ hard and soft acids and bases (HSAB),[62-64] ~~were~~ was calculated to characterize the electrophilic reactivity of chloroethylenes. In order to calculate the ~~electrophilic index (ω)~~, ~~firstly~~ the ~~highest occupied molecular orbital (~~HOMO~~)~~ and ~~lowest unoccupied molecular orbital (~~LUMO~~)~~ energies were calculated at the BSIII level ~~of theory~~ in the gas phase~~.~~, ~~thus~~From these, it is possible to

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Commented [KPK11]: HOMO was already mentioned previously; explain at first incidence

quantify the three basic HSAB parameters as follows: hardness (η) as ($E_{LUMO} - E_{HOMO}$)/2, softness (σ), defined as the inverse of hardness ($1/\eta$), and the chemical potential (μ), as ($E_{LUMO} + E_{HOMO}$)/2. Then, the electrophilic index (ω) was calculated as μ^2/η.

Kinetic Data. The reaction rate constant ~~of~~ for reaction of the chloroethylenes with cob(I)alamin, k, and the corresponding free energy of activation, $\Delta G^{\neq}$, ~~can be~~are ~~converted to each other according to~~related by the Eyring equation (eq 2):

$$k = \frac{k_B T}{h} \cdot \frac{1}{c^0} \exp\left(-\frac{\Delta G^{\neq}}{RT}\right) \quad (2)$$

where k_B is the Boltzmann constant, h is the Planck constant, R is the gas constant, T the temperature in Kelvins, and c^0 is the concentration defining the standard state (typically 1 mol/L). This equation was used to estimate relative rate constants from activation barriers.

Isotope Effects.[65-67] The ~~obtained~~ Hessians obtained from the ~~above mentioned~~ frequency calculations ~~after geometry optimizations~~ were used to calculate kinetic isotope effects (KIEs) ~~with~~ using the ISOEFF package.[68] KIEs were obtained according to the Bigeleisen equation at 298 K for the transition from two separate reactants to the corresponding transition state. The apparent kinetic isotope effects (AKIE) value ~~of~~ from experiments can be approximated ~~from~~ using the bulk isotope fractionation factors (ε_{bulk}) by eq 3:

$$AKIE \approx \frac{1}{1 + n/x \cdot z \cdot \varepsilon_{bulk}} \quad (3)$$

where n is the number of atoms of the considered element, x is the number of atoms of the considered element at the reactive position, and z is number of atoms of the ~~considered~~ element in intramolecular isotopic competition.[33] It should be noted that in this form the secondary isotope effects are neglected, an assumption that ~~should be plausible~~is reasonable for chlorine KIEs.[69]

Commented [KPK12]: Just a point perhaps to mention: These calculations assume 1) Koopman's theorem applies to make HOMO /L UMO resemble IP and EA, and 2) the finite difference approximations to hardness and chemical potential viz. the Mulliken electronegativity

Commented [KPK13]: I assume?

~~All calculations in this work were performed with the Gaussian 09 Revision D.01 program package.~~[70]

Results and Discussion.

Calibration vs. Experimental Data.

~~Based on DFT optimization results with the PBE functional,~~Our DFT computations show that two-electron reduction of the complete model of cob(III)alamin to cob(I)alamin leads to both the axial methyl and DMB groups leaving ~~off, providing~~to produce the 4-coordinated cob(I)alamin species ~~, as shown in~~ **Figure 1**), as expected. The corrin macrocycle in the complete cob(I)alamin model is almost planar with averaged Co-N bond length of 1.86 Å. This is ~~in consistent with~~fits the average Co-N bond lengths of 1.86 and 1.88 Å reported in two recent XAS studies.[53-54] In addition, the calculated standard reduction potential (E^0) value for the cob(II)alamin/cob(I)alamin couple is −0.78 V vs. SCE, near to the corresponding experimental data of −0.85 V vs. SCE,[18] further validating the reliability of the theory level used.

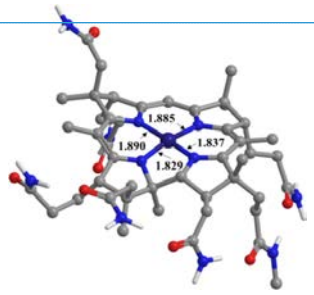

Figure 1. Chemical Structure along with Bond Lengths of Planar Co-N Bonds (Å) for Cob(I)alamin.

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It is not a figure with a lot of information currently and some journals emphasize merging figures with more panels when this happens

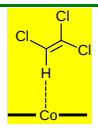
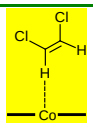
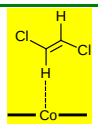
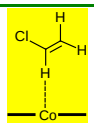
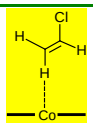
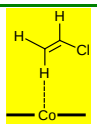
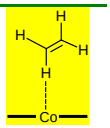
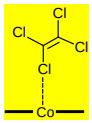
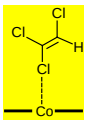
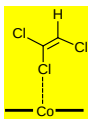
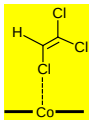
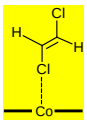
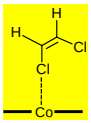
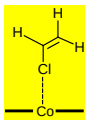
Recently, the dispersion-driven O-H···Pt^{2+} interaction between *trans*-[$PtCl_2(NH_3)$(N-glycine)] and water ~~molecular~~ has been revealed experimentally at very low ~~-~~temperature,[71] ~~which supports~~indicating that the d^8 metal ions in complexes can act as H-bonding acceptors. Considering ~~the fact~~ that the dominant contribution to the ground state is the closed-shell singlet d^8 Co(I) for cob(I)alamin, ~~a scientific question~~the possibility arises ~~that whether~~that ~~r~~ the Co(I)···H interaction between cob(I)alamin and a water molecule ~~molecular~~ can also be formed and consequently influence the cob(II)alamin/cob(I)alamin reduction. ~~Then r~~Relevant ~~computational~~ computations~~calculations have been done,~~[72] ~~which~~ confirm~~s~~ the possibility of forming ~~the~~ a Co(I)···H linkage between cob(I)alamin and water ~~molecular from thermodynamics~~, and ~~suggests that~~ this unusual Co(I)···H interaction may have ~~significant~~ catalytic relevance during the reactivation cycle of ~~the~~ methionine synthase ~~enzyme~~.[73-74] Therefore, the Co(I)···H as well as Co(I)···Cl interactions between cob(I)alamin and chloroethylenes ~~have been~~were explored in this work. As shown in **Table 1**, all Co(I)···H and Co(I)···Cl bond formation processes between cob(I)alamin and different chloroethylenes are endothermic in water solution based on the PBE/BSI free energies including solvation and D3 dispersion corrections, which seem to be nonspontaneous from thermodynamics. This is in accord with previous computations suggesting ~~the earlier computational study~~ that the Co(I)···H bond formation between cob(I)alamin and a water ~~molecular~~ molecule ~~in water solution was~~is not favorable in water solution where water-water inetractions are favored,[72] and ~~-~~ in line with previous experiments indicating ~~al work that there was~~ no such ~~observed~~ H-bonding in water solution.[18] ~~Therefore, this work uses~~We thus used the two separate reactants (i.e., cob(I)alamin and chloroethylene) as starting point to study the reductive dehalogenation mechanisms in the following sections. However, ~~since the~~ earlier ~~computational~~ computations ~~work~~ showed that ~~the nonpolar solvent such as~~ chloroform (a solvent

Commented [KPK15]: Chloroform is polar just not as much as water

~~mimicking of~~with a dielectric constant similar to that of a typical protein environment) ~~made~~ makes ~~the~~ Co(I)···H bond formation ~~process favorable~~feasible, ~~this unusual~~ Co(I)···H or even Co(I)···Cl bonding ~~needs to be~~was considered ~~for~~ here in the context of reductive dehalogenation catalyzed by cob(I)alamin in enzyme.

Commented [KPK16]: Mention where these data are discussed, sounds more like method right now

Table 1. Computed Free Energies (kcal/mol) at the PBE/BSI level of theory with solvation and D3 dispersion corrections for Co(I)···H and Co(I)···Cl Bond Formation between Cob(I)alamin and Chloroethylenes in Water Solution

Co(I)··· H							
ΔG	4.9	4.5	4.0	2.4	4.8	3.3	2.9
Co(I)···Cl							
ΔG	9.7	8.0	9.5	9.1	8.9	9.0	7.3

Outer-Sphere Electron Transfer Mechanism.

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We first~~ly~~ focus on the outer-sphere electron transfer pathway of cob(I)alamin catalyzing chloroethylenes according to eq 4:

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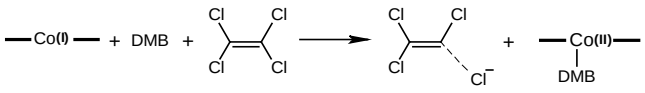

(4)

DMB: 5,6-dimethylbenzimidazole

The free energy barriers of the outer-sphere electron transfer processes ($\Delta G^{\neq}_{ET}$) can be estimated from the Marcus theory[75-78] (calculation details shown in the Supporting Information). The obtained $\Delta G^{\neq}_{ET}$, the free energy of reaction (ΔG_{ET}) ~~as well as~~and the vertical electron affinities (VEA) values for all chloroethylenes are shown in **Table 2**. Both $\Delta G^{\neq}_{ET}$ and ΔG_{ET} values increase in the sequence PCE < TCE < *trans*-DCE < *cis*-DCE < VC, with increasing number of chlorine atoms and decreasing of the ~~vertical electron affinities (~~VEA~~)~~ values. Then a linear free energy relationship (LFER) between $\Delta G^{\neq}_{ET}$ and VEA values for the one-electron-transfer reaction was ~~built~~constructed. ~~As a result, t~~The VEA values ~~are~~ closely correlated ~~to~~ with the free energy barriers with an r^2 of 0.940 ($\Delta G^{\neq}_{ET} = -67.02VEA + 104.11$) (in kcal/mol). Therefore, it is possible to provide a computationally less demanding tool for preliminary evaluation of the free energy barriers of the electron transfer process for cobalamin-mediated reductive dehalogenation of halogenated compounds within one class.

Table 2. The Free Energies (kcal/mol) of the Electron-Transfer Reactions for Cobalamin-Mediated Reductive Dehalogenation of Chloroethylenes, along with the Vertical Electron Affinities (VEA, eV) of Chloroethylenes

	PCE	TCE	*cis*-DCE	*trans*-DCE	VC[a]
VEA	1.36	1.24	1.02	1.13	0.97
$\Delta G^{\neq}_{ET}$	15.9	17.6	34.7	27.3	41.7
ΔG_{ET}	15.6	17.2	31.0	25.7	35.2

[a]VC: vinyl chloride

Note that the outer-sphere electron transfer reactions for all chloroethylenes by cob(I)alamin are highly endergonic~~,~~. ~~however~~However, the calculated standard reduction potentials (E^0) of

vinyl radicals ~~listed in(~~ **Table S3** in the Supporting Information) show that all chlorinated vinyl radicals ~~with~~ have E^0 values between −0.40 V to 0.16 V, i.e. they can be reduced at standard conditions by cob(I)alamin, which could provide additional driving force for the reaction to proceed. The free energy barrier ~~value~~s for PCE and TCE are considerably lower (< 20 kcal/mol), ~~implicating~~ indicating that the outer-sphere electron transfer reactions for cob(I)alamin catalyzing PCE and TCE could take place at normal temperatures, but such reactions are hampered by the high barrier for cob(I)alamin catalyzing *trans*-DCE, *cis*-DCE and VC. These results are consistent with experimental findings~~,~~ that no significant amount of free vinyl radicals were produced in the reactions of cob(I)alamin reducing *trans*-DCE, *cis*-DCE and VC, while significant chlorinated vinyl radicals formed during the reactions of PCE and TCE catalyzed by cob(I)alamin.[22] In the following sections, the competition between the outer-sphere and inner-sphere reduction of chloroethylenes with cob(I)alamin will be addressed.

Inner-Sphere Nucleophilic Substitution Mechanism.

Figure 2 shows the free energy profile for the inner-sphere nucleophilic substitution of PCE with cob(I)alamin, together with geometric details of the relevant molecular species. From separated reactants, cob(I)alamin + PCE, the nucleophilic substitution reaction takes place via the concerted transition state TS_{NS}, associated with a barrier of 14.2 kcal/mol, leading synchronously to base-off trichlorovinylcob(III)alamin ~~under~~ upon loss of ~~chloride anion~~ Cl^- (the Mulliken charge ~~of dissociated~~of Cl changes from 0.11 in the reactants to -0.65 in the products), slightly exothermic of −0.2 kcal/mol relative to reactants. The experimental second-order rate constant of cob(I)alamin-mediated PCE varied slightly with pH (7 – ~9), from 125 ± 7 to 179 ± 10 $M^{-1}s^{-1}$.[21] ~~Thus bringing the kinetic information into~~Using the Eyring equation (eq 2)~~,~~ yields a free energy

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barrier ~~from~~ of 14.3 ~~to~~ 14.6 kcal/mol, very close to the above calculated nucleophilic substitution barrier of PCE with cob(I)alamin. The ~~qualitative~~ comparison of the free energy barriers of nucleophilic substitution ($\Delta G^{\neq}_{NS}$ = 14.2 kcal/mol) and electron transfer ($\Delta G^{\neq}_{ET}$ = 15.9 kcal/mol) ~~estimates the~~suggests a pathway ratio of nucleophilic substitution to electron transfer of ~17:1, in accord with the experimental phenomenon that maximally ~~ten percent~~10% free radicals are formed during dehalogenation of PCE with cob(I)alamin, ~~resulted in the~~i.e. a ratio for inner-sphere pathway to out-sphere pathway of ~~more than~~≥ 10:1.[21] No minima along the reaction path corresponding to initial cob(I)alamin • PCE adduct could be located, and the IRC calculations verified the reaction path leading down from the transition state to separate cob(I)alamin and PCE as reactants (reverse direction) and base-off trichlorovinylcob(III)alamin as product (forward direction), as shown in **Figure S3** in the Supporting Information. ~~The~~ A characteristic S_N2 transition-state geometry, with bond making occurring simultaneously with bond breaking, is ~~easily~~ recognized. This S_N2 transition-state geometry is rather unsymmetrical with the forming C-Co bond (2.05 Å) much shorter than the breaking C−Cl bond (2.40 Å).

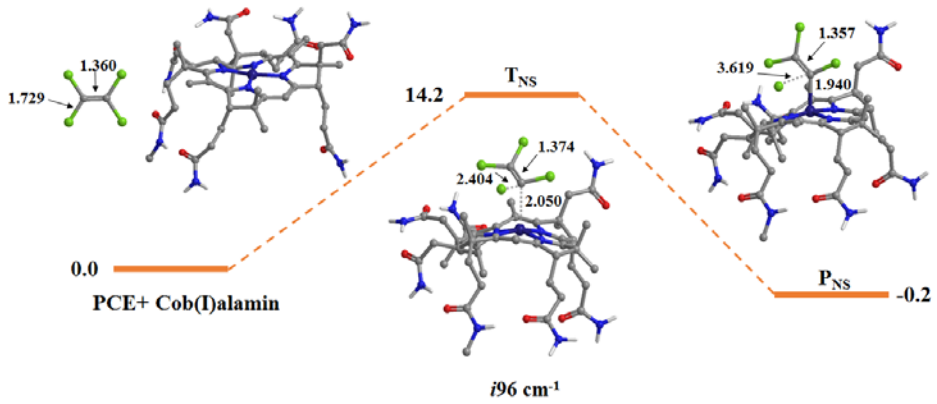

Figure 2. Free Energy Profile (kcal/mol) of Cob(I)alamin-mediated Reductive Dehalogenation of PCE, along with the Optimized Geometries (Å) of the Key Species and the Imaginary Frequency in Transition State in wave numbers. Relative ~~Energies~~ energies were ~~given in the Form of~~ based on PBE/BSI ~~Free~~ free ~~Energies~~ energies with ~~Single~~single ~~Point~~ point ~~Solvation~~ solvation and D3 ~~Dispersion~~ dispersion ~~Corrections~~ corrections ($\Delta G + E_{solv} + E_{disp}$).

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Electrochemical experiments[79] and DFT calculations[80] have demonstrated that the one-electron-reduced alkyl-cobalt complexes exhibit significant lowering of the Co-C bond dissociation energy in comparison to their neutral precursors. Therefore, once the trichlorovinylcobalamin is formed, it is essential to investigate the subsequent reductive cleavage processes of Co-C bond for both the base-off and base-on trichlorovinylcobalamins, to give the dehalogenation product of PCE, *i.e.* TCE. The calculated E^0 for base-off and base-on trichlorovinylcobalamins are −0.63 V vs. SCE and −0.58 V vs. SCE, respectively. In combination with the calculated E^0 of −0.78 V vs. SCE and experimental E^0 of −0.85 V vs. SCE[18] for the cob(II)alamin/cob(I)alamin couple, it demonstrates that both the base-off and base-on trichlorovinylcobalamins ~~could~~ can be readily reduced under the reductive reaction conditions.

Figure 3 shows the optimized structures for both the one-electron-reduced base-off and base-on trichlorovinylcobalamin (trichlorovinylcob(II)alamin). It is remarkable that the DMB base dissociates far away from the cobalt center with Co-N length of 5.0 Å for the base-on trichlorovinylcob(II)alamin, suggesting the base-on trichlorovinylcob(II)alamin probably has both ~~the~~ "base-on" and "base-off" properties. The complete base-on trichlorovinylcob(II)alamin with the loose axial DMB base is quite different from the previously reported simplified base-on trichlorovinylcob(II)alamin with the tight axial imidazole base,[44] partly because DMB is a weaker donor ligand than imidazole and partly due to steric repulsion. Subsequently, cleavage of the Co-

C bond of trichlorovinylcob(II)alamin may occur homolytically to form a vinyl anion and cob(II)alamin, or heterolytically to form a vinyl radical and cob(I)alamin, as shown in **Scheme 3**. The calculated free energies for homolysis and heterolysis of the base-off form are 29.6 and 11.0 kcal/mol, respectively (18.1 and 13.9 kcal/mol without dispersion correction), while the corresponding values for the base-on form are 21.8 and 3.2 kcal/mol (−3.5 and −7.7 kcal/mol without dispersion correction). Thus, the heterolytic cleavage of the Co-C bond of the base-on trichlorovinylcob(II)alamin is the most favorable pathway, which is similar to the previous theoretical examination of the Co-C cleavage of reduced *cis*-dichlorovinylcobaloxime.[48]

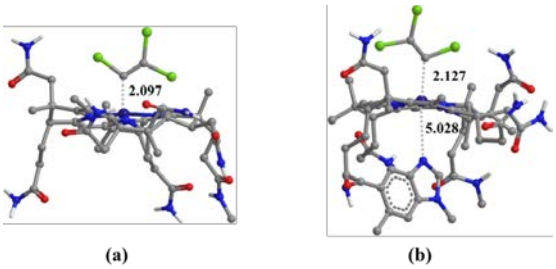
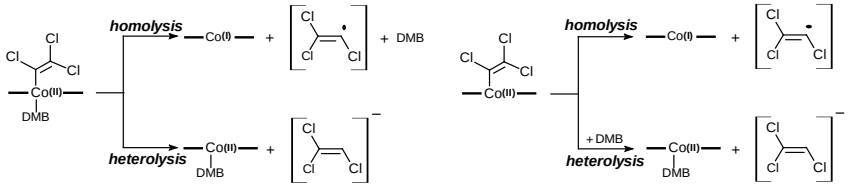


Figure 3. The Optimized Structures of Base-off Trichlorovinylcob(II)alamin (a) and Base-on Trichlorovinylcob(II)alamin (b).

Scheme 3. Homolysis and Heterolysis of the Base-on and Base-off Forms of Trichlorovinylcob(II)alamin



We then investigated the nucleophilic substitution mechanism of TCE, *cis*-DCE, *trans*-DCE and VC. **Table 3** summarizes the relative energies of inner-sphere nucleophilic substitution processes for cobalamin-mediated all chloroethylenes as well as ~~the electrophilic index (ω)~~ for all chloroethylenes. ~~, while T~~The optimized geometries of the S_N2 transition-states are shown in **Figure S1** ~~in the Supporting Information~~. All the reaction paths were verified by IRC calculations ~~that the transition state leading to separate cob(I)alamin and chloroethylene as reactants in the reverse direction and base-off vinylcob(III)alamin as product in the forward direction, as shown in (**Figure S4 ~ S8** in the Supporting Information)~~. ~~And the same as the~~Similarly, for the nucleophilic substitution mechanism of TCE with cob(I)alamin, no initial cob(I)alamin • chloroethylene adduct could be located in the reaction path. ~~It is obvious that~~As seen, the free energy barriers of nucleophilic substitution are generally increasing with decreasing ~~of~~ number of chlorine atoms in chloroethylenes, with an exception of dehalogenation of TCE to *trans*-DCE with larger barriers (minor pathway for dehalogenation of TCE). Moreover, ~~quantitatively,~~ increasing the electrophilic reactivity of chloroethylenes as quantified through ~~the electrophilic index (ω)~~ decreases the nucleophilic substitution barrier, thus yielding a good correlation with an r^2 value of 0.938 ($\Delta G^{\neq}_{NS} = -0.195\omega + 6.95$). ~~The~~ This result indicates the suitability of ω ~~to~~ for screening the reactivity of cobalamin-mediated reductive dehalogenation of halogenated compounds ~~in~~ via the inner-sphere nucleophilic substitution pathway.

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Table 3. The Relative Free Energies (kcal/mol) for Cobalamin-mediated Reductive Dehalogenation of Chloroethylenes during the Inner-Sphere Nucleophilic Substitution Processes along with the Electrophilic Index (ω) of Chloroethylenes

	PCE	TCE[a]	TCE[b]	*cis*-DCE	*trans*-DCE	VC

ω	4.12	3.83	3.83	3.34	3.59	3.12
TS_{NS}	14.2	16.9	18.6	18.2	17.3	19.5
P_{NS}	-0.2	-7.9	-1.8	-5.1	-6.0	-12.5

[a]dehalogenation of TCE to produce *cis*-DCE**;** [b]dehalogenation of TCE to produce *trans*-DCE

~~It is apparent that c~~Conversion of TCE may produce *cis*-DCE and *trans*-DCE, respectively. From the relative energies in **Table 3**, *cis*-DCE is the main product under both kinetic and thermodynamic control. The computational kinetic data from the Eyring equation (eq 2) predicts a preponderance of produced *cis*-DCE over *trans*-DCE by a factor of 18 to 1, which is in excellent agreement with the product~~s~~ distribution of cobalamin-mediated dehalogenation of TCE found experimentally (*cis*-DCE : *trans*-DCE ratios > 15 : 1).[20-21, 23] The energy barrier for conversion of TCE into *cis*-DCE gives ~~the~~ a rate constant of 2.5 $M^{-1}s^{-1}$, almost the same as the experimental data from 2.4 ± 0.2 $M^{-1}s^{-1}$ to 3 ± 0.1 $M^{-1}s^{-1}$.[21] ~~Then, combining with~~Using the above obtained free energy barrier of the electron-transfer process ($\Delta G^{\neq}_{ET}$ = 17.6 kcal/mol) for TCE with cob(I)alamin, the ratio of nucleophilic substitution pathway to electron transfer pathway is predicted to be 3.5 : 1 ~~through~~ based on the Eyring equation (eq 2), consistent with the experimentally determined ratio ~~for inner-sphere pathway to out-sphere pathway~~ of > 2.3 : 1.[21]

Moreover, the reaction barriers of cob(I)alamin-mediated dehalogenation of *cis*-DCE, *trans*-DCE and VC are within 20 kcal/mol.~~,~~ ~~thus~~ Accordingly, the nucleophilic substitution reactions for these less-chlorinated ethylenes by cobalamin could ~~happen in theory~~in principle occur. However, ~~the~~ previous experimental work ha~~s~~d shown that the increase of pH by one unit lead~~s~~ed to a decrease of the reaction rate by roughly a factor of ten, suggesting that a proton ~~was~~ is involved in the rate-determining step,[22] which contradicts the inner-sphere nucleophilic substitution pathway, to be discussed below.

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The results ~~in former section~~above suggest that the trichlorovinylcobalamin~~e~~ could be rapidly reduced under reductive conditions. Therefore, ~~it is necessary to~~we investigated the redox potentials of other less-chlorinated vinylcobalamins to know whether they can be reduced under similar conditions. Due to the ~~preponderance~~ production of more *cis*-DCE ~~over~~ than *trans*-DCE ~~for~~ when TCE reacts with cobalamin, ~~the following work only~~we focused on *cis*-dichlorovinylcobalamin ~~(dichlorovinylcobalamin)~~. As shown in **Table 4**, the calculated E^0 values ~~are becoming~~ become more negative with ~~decreasing of~~fewer chlorine atoms in the chlorinated vinylcobalamins, and the E^0 values for all base-on dichlorovinylcobalamin, *cis*- and *trans*-chlorovinylcobalamin, and vinylcobalamin are more negative than their base-off forms. ~~Especially~~In particular, the E^0 value for the base-off dichlorovinylcobalamin (−0.86 V vs. SCE) is substantially higher than for the corresponding base-on form (−1.23 V vs. SCE), while the E^0 values for the base-off chlorovinylcobalamins and vinylcobalamins are only a bit more positive than for their base-on forms. ~~It is obvious that a~~Among these less-chlorinated vinylcobalamins, only the base-off dichlorovinylcobalamin ~~would appear as~~is thus a candidate for promotion of reduction by cob(I)alamin, although ~~the~~ E^0 ~~value~~ for the base-off dichlorovinylcobalamin (−0.86 V vs. SCE) is a bit more negative than ~~the~~ E^0 ~~value of~~for the cob(II)alamin/cob(I)alamin couple (experimental value: –0.85 V vs. SCE; calculated value: –0.78 V vs. SCE). All other base-off and base-on chlorinated vinylcobalamins are not feasible intermediates due to their much more negative E^0 value than the cob(II)alamin/cob(I)alamin couple.

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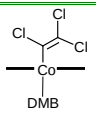
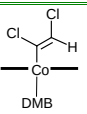
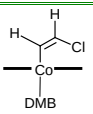
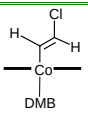
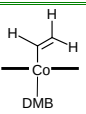
Table 4. Computed Aqueous-Phase Standard Reduction Potentials (E^0) (V vs. SCE) for the Base-off and Base-on Vinylcobalamins

	trichlorovinyl cobalamin	dichlorovinyl cobalamin	*cis*-chlorovinyl cobalamin	*trans*-chlorovinyl cobalamin	vinyl cobalamin

Base off					
E^0	**–0.63**	**–0.86**	**–1.28**	**–1.21**	**–1.40**
Base on					
E^0	**–0.58**	**–1.23**	**–1.39**	**–1.27**	**–1.45**

After formation of the one-electron-reduced base-off form of dichlorovinylcobalamin (dichlorovinylcob(II)alamin), the corresponding base-on form with re-coordination of the DMB base to the Co center may be formed. As shown in **Figure 4**, ~~different from the base-on~~in contrast to trichlorovinylcob(II)alamin, the DMB base coordinates strongly with the cobalt center ~~for the~~ to produce base-on *cis*-dichlorovinylcob(II)alamin. The geometry difference between the base-on trichlorovinylcob(II)alamin and dichlorovinylcob(II)alamin may ~~come~~ arise from the much stronger inductive effect of the trichlorovinyl-fragment (Mulliken charge: −0.71) compared to the dichlorovinyl-fragment (Mulliken charge: −0.44) (more detailed electronic structure analysis is given ~~in the following part~~below). We then calculated the free energy changes of the Co-C bond cleavage for both the base-off and base-on dichlorovinylcob(II)alamin. As for trichlorovinylcob(II)alamin, heterolysis in the base-on forms is the thermodynamically preferred mode of Co-C bond cleavage with a free energy of 7.7 kcal/mol (−7.2 kcal/mol without dispersion correction) (for the detailed free energy comparisons see **Table S26** in the Supporting Information).

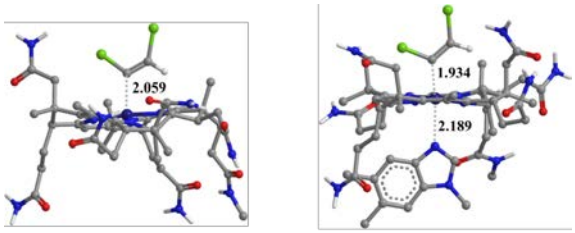


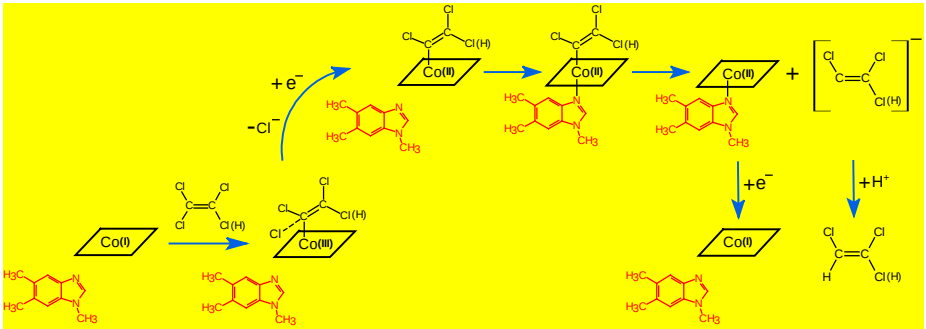
Figure 4. The Optimized Structures of Base-off Dichlorovinylcob(II)alamin (a) and Base-on Dichlorovinylcob(II)alamin (b).

It is noteworthy that detection of trichlorovinylcobalamin has never been successful, but efforts to detect dichlorovinylcobalamin have been feasible.[25] This has been a long-term unsolved mechanistic topic in cobalamin chemistry.[11] As mentioned above, the base-on trichlorovinylcob(II)alamin with quite long Co-N length shown in **Figure 3** is close to its base-off form, making the reduction potential of the base-on trichlorovinylcobalamin (−0.58 V vs. SCE) as negative as the base-off form (−0.63 V vs. SCE), ~~so~~ i.e. the base-on trichlorovinylcobalamin is easily reduced under reductive conditions. By contrast, the tight Co-N bond ~~with bond length of~~(2.2 Å) in the base-on dichlorovinylcob(II)alamin shown in **Figure 4** causes the reduction potential of the base-on dichlorovinylcobalamin (−1.23 V vs. SCE) to be much more negative than its base-off form (−0.86 V vs. SCE), ~~resulting in~~implying a relatively longer lifetime for the base-on dichlorovinylcobalamin. Thus, it is possible to observe the mass consistent with the dichlorovinylcobalamin in mass spectra of the TCE dehalogenation reaction. On the other hand, although the DMB base dissociates far away from the cobalt center for the base-on trichlorovinylcob(II)alamin, the ~~strong~~ destabilization ~~effect~~ by the nitrogen lone pair electrons ~~from the axial~~of DMB ~~ligand~~ results in a weaker Co–C bond for the base-on trichlorovinylcob(II)alamin than its base-off form, and its Co-C bond would be more easily

cleaved. This proposed DMB-dependence mechanism ~~is suggested to~~may be tested by distinct model systems in future experimental work.

Thus, the study suggests that in an inner-sphere nucleophilic substitution pathway of PCE and TCE with cobalamin, *an "on/off" conformational ~~switch~~ change ~~like~~ similar to "ping~~-~~pong" playing is active* (see **Scheme 4**). Specifically, the "base-off" cob(I)alamin facilitates substrate reduction, after which the formed "base-off" chlorinated vinylcobalamins can be readily reduced (the "base-on trichlorovinylcobalamin can be approximately taken as "base off" due to the quite long Co-C bond), then re-coordination of the DMB base provides additional thermodynamic driving force for the heterolytic cleavage of the Co-C bond to complete the overall reaction step.

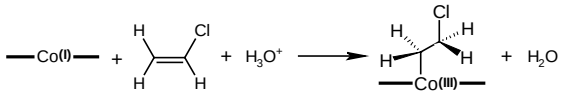
Scheme 4. The Proposed Reaction Pathway for Cobalamin-Mediated Reductive Dehalogenation of PCE and TCE[a]



[a]For cob(II)alamin and base-off tri- and di-chlorovinylcob(II)alamin, the spin is mainly localized on Co (spin density ~~from~~ 0.7 ~~–~~ ~~to~~ 0.9), ~~so the reactions for cob(II)alamin to cob(I)alamin with spin density from nearly one to zero and tri- and di-chlorovinylcob(III)alamin to tri- and di-chlorovinylcob(II)alamin with the~~ spin density ~~from zero to nearly one, really undergo~~changes of ~1 imply metal-centered reductions; Mulliken charge~~s~~ of dissociated Cl changes from 0.07 ~~–~~ ~~~~ 0.11 in the reactants~~,~~ ~~to~~ ~~to~~ (~~–~~0.46) ~~~~ to (~~–~~0.35) in the transition state, and to (~~–~~0.67) ~~–~~ (~~–~~0.65) in the product complex; ~~Mulliken charge of~~ The $CCl_2Cl(H)$ fragment changes ~~from 0.38 ~ 0.35 in the base-off tri-/di-chlorovinylcob(III)alamin, to 0.70 ~ 0.66 in the base-off tri-/di-chlorovinylcob(II)alamin, and to 0.71 ~ 0.44 in the base-on tri-/di-chlorovinylcob(II)alamin~~accordingly (see Supporting Information for more details).

Inner-Sphere Nucleophilic Addition Mechanism

The above ~~work provides the~~discussion derived the reasonable mechanistic reductive dehalogenation pathway of cobalamin-mediated PCE and TCE, ~~,~~ H~~h~~owever, the reductive dehalogenation mechanism for cobalamin-mediated less-chlorinated ethylenes, *cis*-DCE, *trans*-DCE and VC, is still puzzling. Experimental work ~~have~~ has shown that *cis*-DCE, *trans*-DCE and VC were reductively dehalogenated by cob(I)alamin in a pH-dependent ~~mode~~way,[22] suggesting that the initial and rate-determining step is possibly the addition of cob(I)alamin to these less-chlorinated ethylenes with simultaneous protonation. Therefore, the nucleophilic addition pathway is computed for *cis*-DCE, *trans*-DCE and VC according to eq 5 (taking VC as an example):



(5)

The reaction free energies (ΔG_{NA}) for cob(I)alamin-mediated *cis*-DCE, *trans*-DCE and VC during the nucleophilic addition pathway are −51.9 kcal/mol, −52.8 kcal/mol and −48.9 kcal/mol,

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respectively. Thus, ~~the~~ a notable driving force of the nucleophilic addition pathway for all of the less-chlorinated ethylenes with cob(I)alamin to produce corresponding chlorinated ethylcobalamins is evident.

Subsequently, the E^0 and VEA of both the base-on and base-off chlorinated ethylcobalamins were calculated ~~(, as is shown in~~ **Table 5**). Note that the attempts to optimize the one-electron-reduced base-off dichloroethylcobalamin (dichloroethylcob(II)alamin) and chloroethylcobalamin (chloroethylcob(II)alamin) lead directly to the elimination of chloride and formation of VC and ethylene, respectively, so ~~it is not applicable to calculate the~~calculation of E^0 is not reliable~~value~~ for the base-off less-chlorinated ethylcobalamins. ~~At the same time, t~~The E^0 values for the base-on dichloroethylcobalamin and chloroethylcobalamin are −1.19 V vs. SCE and −1.24 V vs. SCE, respectively, much more negative than ~~the~~ E^0 ~~value~~ for the cob(II)alamin/cob(I)alamin couple, i.e. they are ~~difficult~~ hard to ~~be~~ reduce~~d~~. However, the VEA values of the base-off forms are larger than the corresponding base-on forms, so it may be inferred that the formed "base-off dichloro- and chloro-ethylcob(II)alamin" would rapidly decompose into the ~~dehalogenation~~ dehalogenated products. Thus, barring unexpectedly high barriers, the nucleophilic addition with simultaneous protonation for cob(I)alamin-mediated less-chlorinated ethylenes would be favored even in basic solutions with very low concentration of H_3O^+.

~~Experimental work~~Kinetic studies ~~has~~ have shown that VC reacted faster with cob(I)alamin than *cis*-DCE and *trans*-DCE. The significance of this ~~phenomenon~~ is difficult to evaluate, because it is challenging and error-prone to calculate ~~the~~ acidity constants for transition-metal complexes in solution, ~~thereby, to~~and thereby obtain the free energy barriers ~~in the nucleophilic addition pathway~~ with simultaneous protonation ~~is a difficult task~~. Nevertheless, the calculated proton affinity (PA) for *cis*-DCE, *trans*-DCE and VC is 5.4 eV, 5.5 eV and 5.8 eV, respectively, consistent

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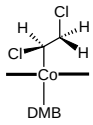
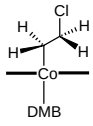
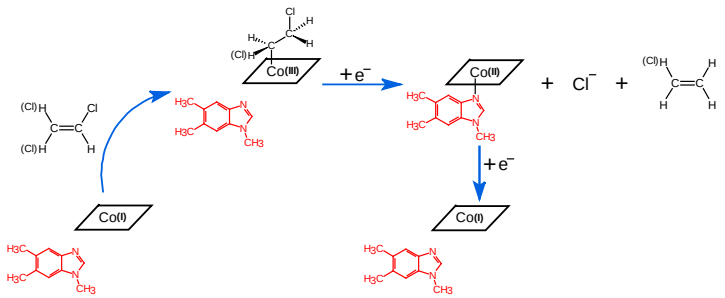
with the order of the experimental kinetic data (*cis*-DCE < *trans*-DCE < VC). Quantitatively, the relationships between the experimental rate constant (log k) at different ~~PH~~ pH values (p~~P~~H = 7, 8 and 9) and PA of the less-chlorinated ethylenes reveal significant correlations between both properties (log k_{pH}~~k~~$_{pH=7}$ = 0.22PA + 5.72, r^2 = 0.986; log k_{pH}~~k~~$_{pH=8}$ = 0.20PA + 5.87, r^2 = 0.994; log k_{pH}~~k~~$_{pH=9}$ = 0.20PA + 6.04, r^2 = 0.997). Therefore, PA ~~would be~~is a good probe for the kinetic information in the nucleophilic addition pathway with simultaneous protonation for cob(I)alamin-mediated halogenated compounds.

Table 5. Computed Aqueous-Phase Standard Reduction Potentials (E^0) (V, vs. SCE) and Vertical Electron Affinities (VEA) (kcal/mol) for the Base-off and Base-on Forms of Ethylcobalamin

	Base-off	E^0	VEA	Base-on	E^0	VEA
dichloroethyl cobalamin		/	70.8		−1.19	64.8
chloroethyl cobalamin		/	65.9		−1.24	60.4

In conclusion, the computations provide support for the mechanistic routes and indicate a distinct type of "*on/off switch*" occurring during cobalamin-mediated reductive dehalogenation of the less-chlorinated ethylenes of the nucleophilic addition pathway: ~~the~~ The initial step is the addition of the "base-off" cob(I)alamin to the less-chlorinated ethylenes with simultaneous protonation.~~,~~ T~~t~~hen the formed base-off form of dichloro- and chloro-ethylcobalamin ~~could~~ can produce the dehalogenation products directly with formation of "base-on" cob(II)alamin under the reductive reaction conditions (see simplified sketch in **Scheme 5**).

Scheme 5. The Proposed Reaction Pathway for Cobalamin-Mediated Reductive Dehalogenation of *cis*-DCE, *trans*-DCE and VC[a]



[a]Mulliken charge ~~of dissociated Cl changes from -0.01 ~ 0.04 in the reactants, to -0.08 ~ -0.05 in base-off dichloroethylcob(III)alamin, and to -1.0 in in the product complex; Mulliken charge of $CH_2Cl(H)$ fragment changes from -0.04 ~ 0.01 in the reactants, to -0.17 ~ -0.08 in the base-off tri-/di-chlorovinylcob(III)alamin.~~s can be found in Supporting Information.

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Electronic Structure ~~Characteristics~~Analysis.

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The unique nature of the C−Co−N bonding in cobalamin, with the competing σ and π effect~~s~~s, ~~has continued to be~~is an important mechanistic subject. **Figure 5** shows the ~~importantly~~ relevant frontier molecular orbitals during the reductive processes of chlorinated vinylcobalamins ~~(, while~~ the relevant frontier molecular orbitals for chlorinated ethylcobalamins are shown in **Figure S2** in the Supporting Information~~)~~. As shown in **Figure 5 (a)**, the ~~lowest unoccupied molecular orbitals (~~LUMOs~~)~~ of base-off tri- and di-chlorovinyl cob(III)alamins are largely associated with the $\sigma_{Co\text{-}C\alpha}$* orbital, whereas base-off non-chlorovinyl cob(III)alamins have LUMOs mainly coinciding with the corrin macrocycle π* orbital, and the LUMO of base-off mono-chlorovinyl cob(III)alamin is mixed corrin macrocycle π* (major) and $\sigma_{Co\text{-}C\alpha}$* (minor)~~ orbital~~. Interestingly, the mixed

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character ~~for~~ of the LUMOs of base-off mono-chlorovinyl cob(III)alamin resembles a transition between LUMOs of base-off tri/di-chlorovinyl and non-chlorovinyl cob(III)alamins. The significant difference in LUMO character is mainly due to the fact that the nitrogen atoms of the corrin ring of cobalamin are more electron-rich than the mono- and non-chlorinated ethylene fragments (Mulliken charge: −0.25 ~~~ to −0.17) compared with the tri- and di-chlorovinyl ethylene fragments (Mulliken charge: −0.38 ~~~ to −0.33), so that the corrin ring has larger overlap with the relevant d-orbital in Co than with the C_α atom in the ethylene fragment resulting in dominant π* orbital for LUMOs of mono- and non-chlorinated cob(III)alamins (E_{LUMO}: −5.05 ~~~ to −5.10 eV). By contrast, the strong inductive effect of tri- and di-chlorinated ethylene fragment withdraws electron density from the Co center to reduce the effective nuclear charge of the metal ion, thereby lowering the energies of Co d_{z^2} orbitals and undergoing a large stabilization of LUMOs on the $\sigma_{Co\text{-}C\alpha}$* orbital for base-off tri- and di-chlorovinyl cob(III)alamins (E_{LUMO}: −5.66 ~~~ to −5.38 eV). Generally speaking, the ~~contribution of~~ electron-withdrawing inductive effects from ~~more than or equal to two~~two or more electronegative chlorine atoms ~~is able to~~can lower the LUMOs of base-off chlorinated vinylcobalamins, thus producing the corresponding lower E^0 values shown in **Table 4**.

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Higher potentials imply easier to reduce = lower LUMO energy.
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These LUMOs of base-off chlorinated vinylcob(III)alamins are initially occupied upon one-electron reduction to produce the corresponding single occupied molecular orbitals (SOMOs). As shown in **Figure 5 (b)**, these SOMOs remain ~~the same as the localization of their precursor LUMOs~~unchanged as reflected also in~~from~~ the spin densities (ρ) of ~~, that such values are~~ 0.98 ~~–~~~0.99 for SOMOs on the $\sigma_{Co\text{-}C\alpha}$* orbital of tri- and di-chlorovinyl cob(II)alamins~~,~~ and ~~are~~ 0.87 ~~–~~~0.88 for the corrin macrocycle π* ~~orbital~~ of mono- and non-chlorinated vinylcob(II)alamins. After recoordination of the DMB base, most SOMOs coincide with the corrin

macrocycle π* orbital of base-on cob(II)alamins~~:~~ ~~particularly t~~The SOMO localization changes from $\sigma_{Co\text{-}C\alpha}$* orbital-like (ρ = 0.98) in the base-off dichlorovinylcobalamin to corrin π* orbital-like (ρ = 0.88) in the base-on species. The reason for this major electronic structure change from base-off to base-on dichlorovinylcobalamin is that an additional strong σ-antibonding between Co and the DMB base in the base-on species leads to a considerable destabilization of the $\sigma_{Co\text{-}C\alpha}$* orbital, thus resulting in SOMO with considerable corrin π orbital character. However, the strong withdrawing inductive effect ~~from~~ of the three chlorine atoms in base-on trichlorovinylcobalamin ~~is able to~~ cancels out the σ-donating effect ~~from the~~of DMB ~~base th~~rough still reducing the effective nuclear charge of Co ~~atom~~ to lower the energy of its d_z^2 orbital, thereby retaining SOMO localization on the $\sigma_{Co\text{-}C\alpha}$* orbital and repelling the DMB base. Interestingly, the distant DMB base in base-on trichlorovinylcobalamin shows weak Van der Waals attraction to further lower the SOMO $\sigma_{Co\text{-}C\alpha}$* orbital, as reflected in comparison of SOMO energies of base-off trichlorovinylcobalamin (E_{SOMO} = −2.92 eV) and base-on trichlorovinylcobalamin (E_{SOMO} = −3.07 eV).

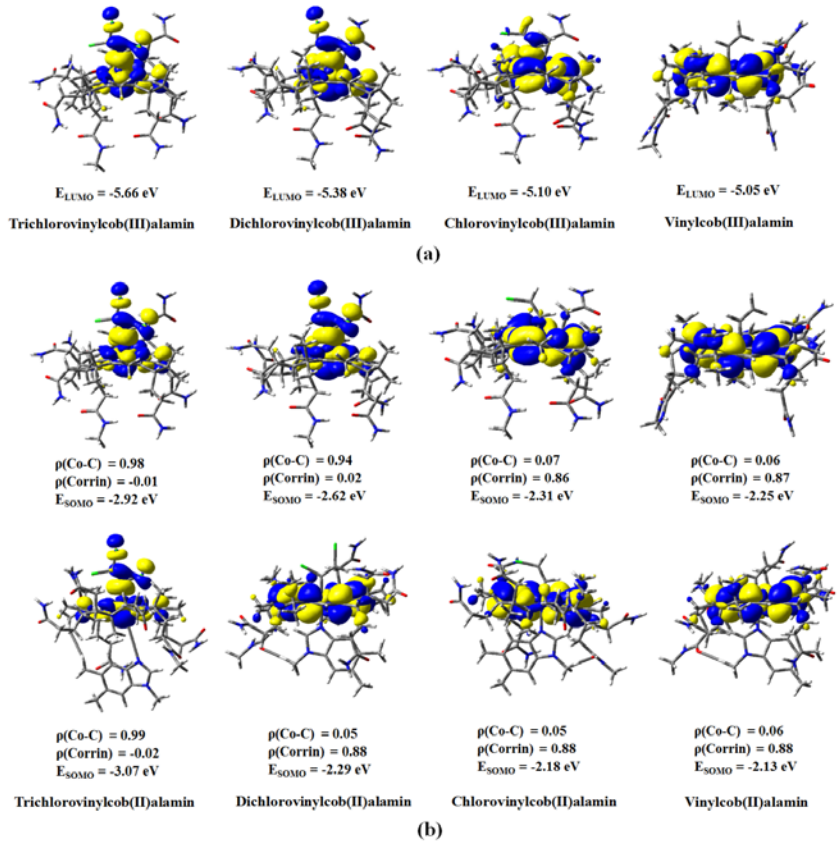

Figure 5. (a) Side View of the LUMOs in the Base-off Chlorinated Vinylcob(III)alamins along with the LUMO Energies (E_{LUMO}); **(b)** Side View of the SOMOs in the Base-off and Base-on Chlorinated Vinylcob(II)alamins along with the SOMO Energies (E_{SOMO}) as well as Spin Density for Co-C Bond [ρ(Co-C)] and Corrin Macrocycle [ρ(Corrin)].

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Isotope Effects.

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Table 6 summarizes the calculated average carbon KIE (KIE_C) values during the inner-sphere nucleophilic substitution pathway for cobalamin-mediated chloroethylenes, together with the experimental ε_{bulkC} as well as the compound average carbon AKIE ($AKIE_C$) obtained from ε_{bulkC} according to eq 3. The similarity of the experimental $AKIE_C$ values ~~with~~ and computational KIE_C values confirm that cobalamin-mediated PCE and TCE dehalogenation proceeds through the nucleophilic substitution pathway. However, the much larger experimental $AKIE_C$ values ~~than the~~ vs. KIE_C values ~~from~~ for the nucleophilic substitution pathway for *cis*-DCE and VC~~,~~ supports the nucleophilic addition of cob(I)alamin to one of the carbon atoms of these chloroethylenes and simultaneous protonation of the other carbon atom, because only the concerted reactions could increase the kinetic isotope effects to the ~~most~~ full extent.

Table 6. Average Calculated Carbon Kinetic Isotope Effects (KIE_C) on the Inner-sphere Nucleophilic Substitution Pathway for Cobalamin-mediated Chloroethylenes, as well as the Experimental Carbon Bulk Isotope Fractionation Factors (ε_{bulkC}) and the Compound Average Carbon Apparent Kinetic Isotope Effect ($AKIE_C$)

	PCE	TCE	*cis*-DCE	*trans*-DCE	VC
KIE_C	1.028	1.032	1.026	1.030	1.027
ε_{bulkC}	−15.8‰	−16.1‰	−25.5‰	/	−31.1‰
$AKIE_C$	1.033	1.033	1.054	/	1.066

Most previous work has focused on stable carbon isotopes to study the transformation process of organic pollutants. However, chlorine ~~also~~ has ~~high~~ major relevance ~~for~~ as constituent of many pollutingenvironmental compounds. In practice, only a few chlorine isotope analyses have been performed to investigate the transformation of compounds, and the first~~ly~~ reported chlorine bulk isotope fractionation factor for cobalamin-mediated chloroethylene ~~is~~ was −4.0‰ for TCE,[9] which

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can be converted into $AKIE_{Cl}$ ~~of ~~ 1.012 by eq 3. The calculated average chlorine KIE for cobalamin-mediated TCE in the inner-sphere nucleophilic substitution pathway is 1.009, quite close to the experimental $AKIE_{Cl}$.

~~As shown in this work, t~~The calculations cannot reproduce very precisely the experimental data in this instance, since the latter may be masked. Dual element isotope analysis has attracted considerable interest, the advantage of which is that different mechanisms may be discerned simply by correlating the isotope fractionation factor (ε) ratios for the two elements. Herein, we extend the dual element isotope analysis ~~only~~ based only on experimental data in~~to~~ a new manner for comparison between computations and experiments ~~through~~ by correlating the ratios of ε or KIE for the two elements. ~~Taking~~ As an example, we consider the reaction of cobalamin-mediated TCE with available dual element isotope~~s as an example~~: ~~the~~ The reverse eq 3 yields ~~the~~ computational ε_C of −15.5‰ and ε_{Cl} of −3.0‰~~,~~ thus the ratio of computational ε_C to ε_{Cl} in the nucleophilic substitution pathway is calculated to be 5.1 : 1.0, while the ratio of experimental ε_C (−16.1‰) to ε_{Cl} (−4.0‰) is 4.0 : 1.0, ~~so there is some degree of~~implying some difference between computations and experiments ~~based on the correlating ratio of ε.~~ ~~In the meanwhile~~Meanwhile, the calculated ratio of $AKIE_C$ to $AKIE_{Cl}$ is 1.02~~,~~ while the ratio of computational KIE_C to KIE_{Cl} in the nucleophilic substitution pathway is 1.02 as well. Furthermore, the latest reported experimental work concerning combined carbon and chlorine isotope analysis during the reductive dehalogenation of TCE by cobalamin provides an ε_C value of −15.0‰ (AKIE = 1.031) and an ε_{Cl} value of −3.2‰ (AKIE = 1.010),[31] which can be converted into $AKIE_C/AKIE_{Cl}$ of 1.02 again, although ~~there is some difference between~~the two previous studies ~~for experimental ε_C and ε_{Cl} values~~differ somewhat. ~~Since~~ With the most plausible nucleophilic substitution mechanism for cobalamin-mediated TCE ~~is~~ outlined above, the comparison between correlating ratios of KIE for

the two elements ~~may~~ provides a new probe to detect the reaction mechanism. This approach holds promise to bridge a gap between computations and experiments, because various factors mask the AKIEs of both elements to the same extent so that correlating ratios of KIE for the two elements remain the same.

Previous studies have indicated ~~the~~ ~~variability~~ variable ~~in~~ isotope fractionation of PCE and TCE by a ~~variety of~~different microorganisms[81] probably due to the complex enzymatic environment ~~for~~ of the dehalogenating strain., ~~thus~~ Because of this, the elucidation of biological dehalogenation mechanisms may be hampered ~~by the variability in isotope fractionation~~. In future work, the reaction mechanisms of the biological dehalogenation ~~could~~ is envisioned to be diagnosed ~~potentially~~ by quantum mechanical/molecular mechanical (QM/MM) or cluster modelling to simulate the enzymatic environment in combination with ~~the~~ our new proposed method of relating calculated KIE_C/KIE_{Cl} ~~vs~~ and experimental $AKIE_C/AKIE_{Cl}$.

Conclusions.

This work shows how computational chemistry closes some key ~~unsolved~~ research gaps ~~for~~ relating to cobalamin-mediated reduction of chloroethylenes by distinguishing different mechanisms (**Scheme 2**). To this end, we have developed some useful quantitative methods that rationalize reactivity by: (i) serving as screening tools for predicting the reductive dehalogenation reactivity ~~in~~ of a given mechanism (e.g. *electron affinity* for electron transfer, *electrophilic index* for nucleophilic substitution, and *proton affinity* for nucleophilic addition); (ii) providing *standard reduction potentials* (E^0) of formed chlorinated-cobalamins as ~~one~~ an important parameter ~~for~~ determining the feasibility of the inner-sphere pathway; (iii) ~~offering~~ suggesting the *calculated* KIE_C/KIE_{Cl} *vs experimental* $AKIE_C/AKIE_{Cl}$ as a probe for diagnosing the overall reaction mechanism. ~~Thus, t~~These quantitative methods may be useful ~~for~~ in ~~determination~~ determining ~~of~~ the environmental fate and develop~~ment of~~ing remediation pathways of halogenated organic pollutants.

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Recently, Payne *et al.* proposed a third type of mechanism ~~alternative route~~, with the cobalamin of reductive dehalogenase able to abstract bromide from 2,6-dibromophenol with formation of a Co−Br bond, consequently leading to a C−Br bond cleavage concomitant with protonation of the leaving group by nearby residue Tyr426.[82] At the same time, Bommer *et al.* further emphasized the role of strictly conserved Tyr246 in reductive dehalogenase pointing with its phenolic group toward carbon to donate the proton to neutralize the dichlorovinyl anion formed upon reaction of TCE with cobalamin cofactor.[83] The more detailed reaction mechanism of the new proposed dehalogenation mode was computationally elucidated by Liao et al.[84-85] This new paradigm implies that the repertoire of reductive dehalogenation originating from cobalamin in dehalogenase is even more diverse than previously anticipated, which may guide further modification strategies for cobalamin to mimic the enzyme behavior in *in vitro* reductive dehalogenations. Together with these recent mechanistic findings, our work spans the so far known possible reactivity space of cobalamin in degradation of halogenated compounds, although we expect that further studies are needed to define the relevance of each reaction type to specific conditions and environments.

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ASSOCIATED CONTENT

Supporting Information. Full citation for reference 70; estimation of activation barriers for electron transfer processes by Marcus theory; computed aqueous-phase standard reduction potentials of vinyl radicals; optimized geometries of S_N2 transition-state of cobalamin-mediated dehalogenation of chloroethylenes; frontier molecular orbitals for less-chlorinated ethylcobalamins; experimental rate constants for reaction of less-chlorinated ethylcobalamins with

cob(I)alamin; energies for all molecular species; Mulliken charges and spin densities; intrinsic reaction coordinate (IRC) for verifying transition states; geometrical comparison ~~between~~ of PBE/BSI optimized structures in the gas phase and PBE-D3-CPCM/BSI optimized structures; ~~final~~ one ~~-~~electron symbolic density matrix of complete cob(I)alamin from CASSCF calculations; ~~cartesian~~ Cartesian coordinates of all molecular structures ~~discussed in this work~~. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

*(L.J.) E-mail: jilienv@zju.edu.cn

*(K.P.K.) E-mail: kpj@kemi.dtu.dk

*(P.P.) E-mail: piotr.paneth@p.lodz.pl

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENT

This work was supported by the National Natural Science Foundation of China (21677125). The China National Supercomputing Center in Shenzhen is acknowledged for providing the Gaussian 09 package and the high-performance computing clusters.

ABBREVIATIONS

DFT, density functional theory; DMB, 5,6-dimethylbenzimidazole; PCE, perchloroethylene; TCE, trichloroethylene; *cis*-DCE, *cis*-1,2-dichloroethylene; *trans*-DCE, *trans*-1,2-dichloroethylene; VC, vinyl chloride; IRC, intrinsic reaction coordinate; CSIA, compound specific isotope analysis; KIEs, kinetic isotope effects; AKIEs, apparent kinetic isotope effects; PBE, Perdew-Burke-Ernzerhof; ZPE, zero-point energy correction; CPCM, COSMO continuum-solvation model; SCE, standard calomel electrode; VEA, vertical electron affinities; HSAB, hard and soft acids and bases; HOMO, highest occupied molecular orbital; LUMO, lowest unoccupied molecular orbital; LFER, linear free energy relationship; PA, proton affinity.

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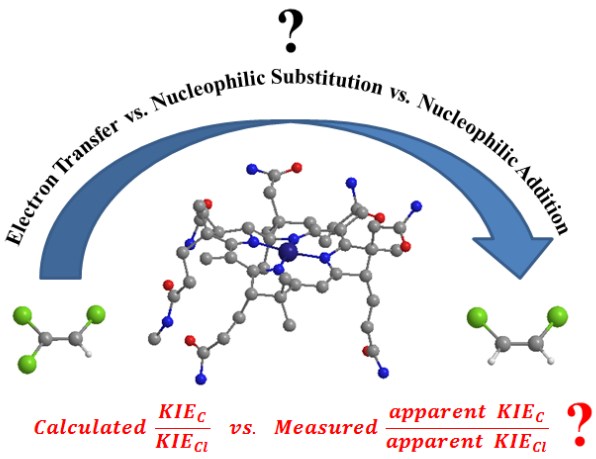
SYNOPSIS


?
Electron Transfer vs. Nucleophilic Substitution vs. Nucleophilic Addition
Calculated $\frac{KIE_C}{KIE_{Cl}}$ vs. Measured $\frac{apparent\ KIE_C}{apparent\ KIE_{Cl}}$?

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